SPAWNING AND EARLY DEVELOPMENT OF *CORBICULA FLUMINEA* (BIVALVIA: CORBICULIDAE) IN LABORATORY CULTURE

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ABSTRACT

The Asiatic clam, *Corbicula fluminea* (Müller), was maintained on the estuarine diatom *Skeletonema costatum* (Greville) in a recirculating aquarium system at 24 to 25°C. Salinity varied from 0 to 8 ppt. Live weight of *C. fluminea* increased from 3% to 179% of initial weight during four months of laboratory culture. The animals then spawned; sperm were ejected out of the exhalent siphons and fertilized eggs were retained in the gills. The first three zygotic divisions occurred 1, 3, and 5 hours after spawning (sperm release), and trochophore larvae developed after 14 hours. Pediveligers were released from parent clams in 4 to 5 days, and metamorphosed to juveniles about 12 hours later. Studies with fluorescent latex microspheres indicated that released larvae were ingesting suspended particles, but brooded larvae were not. Parental broodstock continued to grow under laboratory conditions, and six months after the spawning event, gonad smears of brood-stock revealed gametogenesis taking place.

The exotic bivalve *Corbicula fluminea* (Müller) was first identified in the United States in 1938 (Burch, 1944) and is now widely distributed throughout the country (McMahon, 1982; Counts, 1983). *C. fluminea* has become a pest organism because of biofouling in water treatment facilities, irrigation systems, and power generating stations. Aspects of the reproduction of *Corbicula* spp. have been described (Fuziwara, 1975, 1977, 1978; Kraemer, 1977, 1978, 1980; Kraemer and Lott, 1977; Lee and Chung, 1980; Morton, 1982; Maru, 1981), but difficulties in conditioning and spawning *Corbicula* spp. in the laboratory have hindered detailed examinations of embryogenesis. Sinclair and Isom (1963) were able to maintain *Corbicula* from Tennessee in the laboratory but did not describe growth or spawning of laboratory-cultured animals. Fuziwara (1978) observed ovulation of *Corbicula leana* in outdoor culture ponds, but did not overtly condition the animals prior to spawning, or describe early developmental stages.

General descriptions of larval development of *Corbicula* spp. have been reported (Villadolid and del Rosario, 1930; Cahn, 1951; Sinclair and Isom, 1961, 1963; Britton and Morton 1982), however, most of the illustrations are generalized, and many reports inadequately depict different larval stages. Villadolid and del Rosario (1930) illustrated the larval development of *Corbicula manilensis* from the Philippine Islands, but did not discuss the trochophore larvae. Cahn (1951) described the marsupial trochophores and straight-hinged larvae of *Corbicula leana* from freshwater habitats in Japan. Development of Tennessee populations of *Corbicula* illustrated by Sinclair and Isom (1963) included brief descriptions of trochophores, planktotrophic and benthic veligers. Britton and Morton (1982) discussed and illustrated larval forms of *C. fluminea*, including the marsupial trochophore and veliger larvae.

This paper describes the laboratory culture, spawning, larval development, and larval feeding activity of *C. fluminea*, and compares the results with those of other observers.

TAXONOMY

Bivalves in the genus *Corbicula* von Mühlfeld in the United States have been referred to the taxa *Corbicula fluminea* Müller, *Corbicula leana* Prime, and *Corbicula manilensis* Philippi. Hillis and Patton (1982) presented electrophoretic evidence that two species of *Corbicula* may be present in the United States, but the species question is still under dispute (see, for example Britton and Morton, 1979). Hillis and Patton (1982) recognized two morphological types based on internal shell color (white or purple; the white color form designated *C. fluminea*) and external annulation frequen-

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cy. Our specimens were similar to the white morphotype of Hillis and Patton (1982), and we refer them to the taxon *C. fluminea*.

METHODS

ALGAL CULTURE

Algae that was cultured for feeding clams included the marine flagellate *Isochrysis* aff. *galbana* (Parke), clone T-ISO, the estuarine diatom *Skeletonema costatum* (Greville) Cleve, and several unidentified freshwater algae. T-ISO was obtained from Dr. K. Haines at the University of Texas laboratory, St. Croix, Virgin Islands. Stock cultures of *S. costatum* were obtained from R. R. Guillard, Woods Hole Oceanographic Institution, and *E. gracilis* was obtained from the American Type Culture Collection, Rockville, Maryland. The unidentified freshwater algae mixture was cultured from soil-extract from Lewes, Delaware. Both freshwater and marine cultures were enriched with a modified formulation of f/2 nutrient medium (Guillard and Ryther, 1962; Bolton, 1982).

T-ISO was grown in the laboratory following procedures for marine algal culture described by Bolton (1982). *E. gracilis* and the unidentified algal mixture were grown in freshwater using similar procedures. *S. costatum* was cultured in the same manner as T-ISO, but salinity was reduced from 30 ppt to 5 ppt in stages during culturing; salinity was initially reduced from 30 ppt to 15 ppt, and after two days, salinity was further decreased to between 5 and 8 ppt. Algal cultures at 5 to 8 ppt salinity were harvested semi-continuously for three to five days, and then discarded.

CONDITIONING AND GROWTH OF ADULT *C. FLUMINEA*

Approximately 300 specimens of *C. fluminea* having initial live weights ranging from 1 to 10 g were collected on 9 July 1983 from a freshwater tributary of the Nanticoke River, Nanticoke Wildlife Refuge, Laurel, Sussex County, Delaware, U.S.A. Total live weight of clams was about 1200 g.

For the first two months of laboratory conditioning, clams were maintained in a 200 to 300 *l* recirculating aquarium system at 21°C and fed a mixture of algae including 25 to 50 *l*/day each of T-ISO, *E. gracilis*, and the mixed culture of unidentified freshwater algae. Cell concentrations of algal cultures were 2 to 3 x 10^6 cells/ml for T-ISO, and 1 to 3 x 10^6 cells/ml for *E. gracilis* and the freshwater algae. Algal concentration in the recirculating system ranged from 125 to 750 cells/ml. Aquarium water was drained and replaced with freshwater every 2 to 3 days, and salinity varied from 0 to 5 ppt.

Because the live weight of *C. fluminea* did not increase substantially during the first two months of culture, water temperature in the recirculating system was increased to 24 to 25°C and the diet was changed to 180 *l*/day of *S. costatum* (cell concentration of culture was 0.25 to 1.5 x 10^6 cells/ml). Final salinity of *S. costatum* cultures ranged from 5 to 8 ppt, and salinity of water in the recirculating system varied from 0 to 8 ppt.

Growth of adult clams was monitored by measuring live weight of two groups of clams throughout laboratory culture. Clams were labeled with numbered plastic tape and weighed every 2 to 4 weeks. Group 1 contained 17 clams having initial live weights ranging from 0.81 to 9.14 g. Clams in Group 1 were weighed every week for the first two months of laboratory culture and at monthly intervals thereafter for 205 days. Group 2 contained clams having similar initial live weights (1.60 to 2.78 g). Samples of 22 to 30 clams from Group 2 were weighed monthly from day 67 of laboratory culture to day 298.

The dry meat condition index (after Walne and Millican, (1975) was determined for a sample of 20 clams before laboratory culture and for a sample of 18 clams after one year of laboratory culture. Tissue and shell from each clam were separated and dried for 24 to 48 hours at 60°C, then weighed. The condition index was then calculated by the formula

$$\frac{\text{dry tissue weight}}{\text{dry shell weight}} \times 1000$$

SPAWNING AND LARVAL DEVELOPMENT

Observations of spawning (sperm release) and development of brooding larvae were conducted four hours after aquarium water at 25°C and 8 ppt was drained, clams sprayed vigorously with 19°C fresh water, and the aquarium refilled with 19°C water at 0 ppt salinity. Larval development, therefore, occurred at water temperatures between 19 and 25°C. Salinity ranged from 0 to 8 ppt during the brooding period following daily algae feedings.

When release of sperm was first observed, gametes were filtered from aquarium water, then stained with acridine orange and observed using epifluorescence microscopy. Gills from one or two parent clams were removed approximately every 1 to 3 hours, and embryos were gently teased from the gills into Petri dishes containing freshwater. Early cell divisions were microscopically examined using embryos freshly removed from parental gills and embryos that were in Petri dishes for up to three hours. The time sequence of successive larval stages was determined by noting the time to the nearest hour after sperm release that each stage was first observed.

Released pediveliger larvae were collected from the bottom of the recirculating reservoir by sequentially filtering water with 212 μm, 125 μm, and 75 μm metal sieves. Most larvae were retained on 125 and 75 μm sieves. Pediveligers were transferred to a 16 *l* aquarium having a sand substratum, and fed 1 to 2 *l* *S. costatum* daily. Water in the 16 *l* aquarium was replaced with freshwater every two days. Shell lengths of 25 to 50 pediveligers were measured weekly to monitor growth.

FEEDING ACTIVITY OF LARVAE

The feeding activity of brooded and released larvae was studied using "Fluoresbrite" fluorescent latex microspheres (Polysciences). Microspheres 3.6 μm in diameter had a maximum excitation wavelength of 540 nm, and were

yellow-green in color when examined using epifluorescence microscopy.

Adult clams that were brooding larvae as well as released pediveliger larvae were exposed to algae and microspheres for 6 hours. Algal concentration in the medium was 5 x 10^4 cells/ml and concentration of microspheres was 2.5 x 10^5 spheres/ml. Brooding larvae were removed from the gills of parent clams after exposure to the microspheres and examined using epifluorescence microscopy to qualitatively assess whether or not microspheres had been ingested and were present in the body. Released pediveligers were also examined for fluorescent particles.

RESULTS

GROWTH AND CONDITIONING OF ADULT *C. FLUMINEA*

All clams monitored for growth increased in live weight during laboratory culture. Increase in live weight for clams from Group 1 (initial live weights 0.81 to 9.14 g) ranged from 3% of initial live weight (Fig. 1, clam 16) to 179% of initial live weight (Fig. 1, clam 1). A paired t-test on the initial and final live weights of clams in Group 1 demonstrated that increase in live weight was significant ($t = 11.280$; $P < 0.001$. Clams in Group 2 increased from 2.09 g, standard deviation (s.d.) 0.45 g (Fig. 2) over 164 days of laboratory culture; an increase of 188%. Increase in live weight was significant at $P < 0.001$ (Two-sample t-test; $t = 15.348$).

The condition index of clams after one year of laboratory culture increased significantly, from 67 (s.d. +/– 10, N = 20) at the beginning of laboratory culture, to 115 (s.d. +/– 11, N = 18), after one year of culture (two-sample t-test; $t = 14.085$; $P < 0.001$). This result indicates that increase in live weight of laboratory-cultured clams was due in part to tissue growth, and not due to shell growth alone.

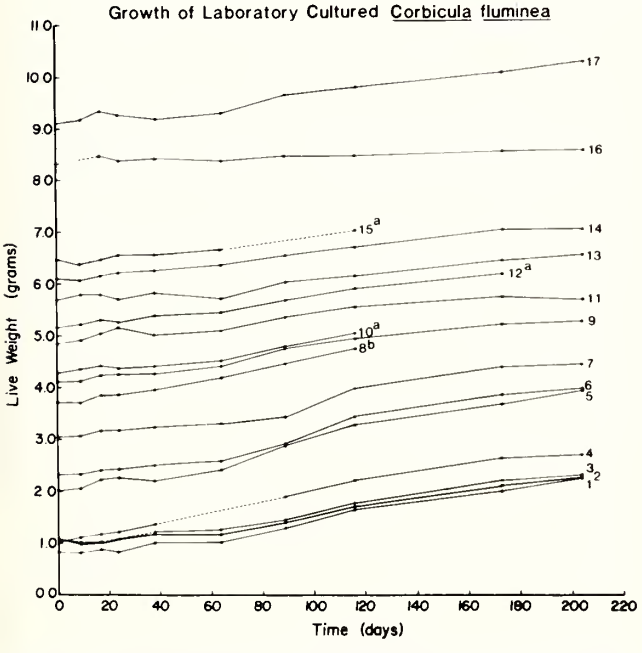

Fig. 1. Live weight of individual *C. fluminea* from Group 1 (initial live weights 0.81 to 9.14 g) during 205 days of laboratory culture. **a.** Label came off clams. **b.** Clam died.

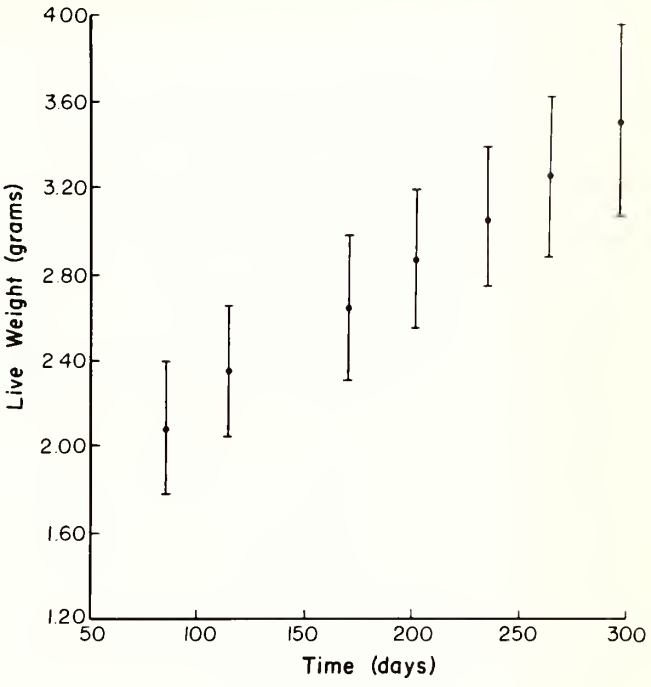

Fig. 2. Mean live weights and standard deviations for 22 to 30 clams from Group 2 (initial live weights 1.60 to 2.78 g) from day 67 to day 298 of laboratory culture.

SPAWNING AND LARVAL DEVELOPMENT

Spawning occurred on 5 November 1983, after four months of laboratory conditioning. Sperm were ejected from the exhalent siphons of adult clams in short bursts. Sperm heads were approximately 16 μm linear distance from end to end (Fig. 3) and bore two flagella. Egg cells, 120 to 170 μm in diameter, were held on the inner demibranchs of the gills of parent clams, and were surrounded by fertilization membranes. Cell counts of gametes filtered from aquarium water during spawning revealed 7.7 x 10^6 sperm cells per ml and only 7 eggs per 500 ml; thus release of eggs by parent clams was negligible, suggesting that fertilization occurred within the clams.

Early cell divisions. The first cell division began about 1 hour after spawning, and the 2-cell stage was complete after 2 hours. The first cell division produced similar sized blastomeres, but in subsequent divisions, cleavage was unequal. The 4-cell to 8-cell stages were first observed 3 and 5 hours after spawning, respectively. Blastulae were first observed 7 hours after spawning, and gastrulation began after approximately 9 hours, at which time the embryo became flattened and developed lobes lateral to the flattened side. Brooding embryos and larvae on the gills of parent clams were encased in a gelatinous envelope that was retained throughout the brooding period (Fig. 4).

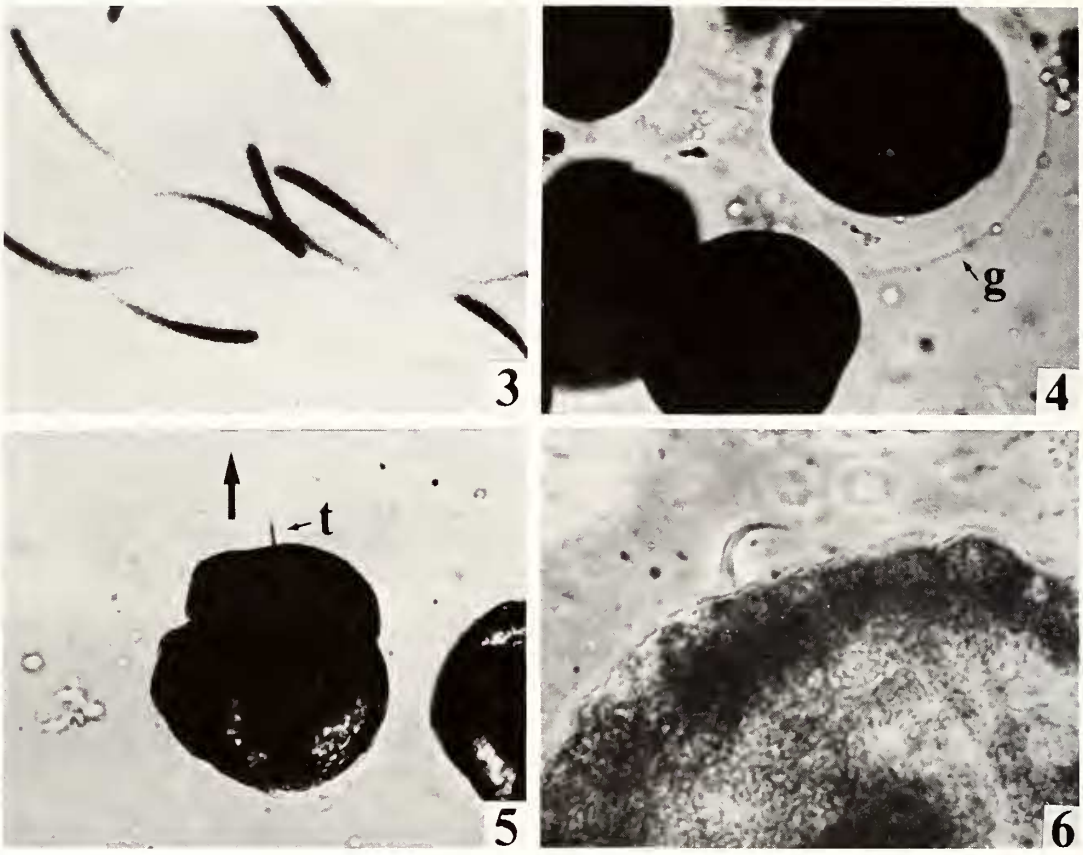

Fig. 3. Sperm of *C. fluminea* filtered from aquarium water and stained with acridine orange. Horizontal field width = 50 μm. **Fig. 4.** Early embryo of *C. fluminea* surrounded by gelatinous envelope (g). Horizontal field width = 400 μm. **Fig. 5.** Trochophore larva of *C. fluminea* about 20 hours after spawning. Larva was removed from gills of parent clam, liberated from gelatinous envelope and suspended in water. Arrow indicates direction of movement. a = apical tuft. Horizontal field width = 150 μm. **Fig. 6.** Movement of the apical tuft of *C. fluminea* trochophore. Horizontal field width = 55 μm.

Trochophores. Early trochophore larvae developed after 14 hours (Fig. 5). Cilia were not evident at 14 hours on trochophores that were removed from parental gills, liberated from the gelatinous envelope, and suspended in water, although particles moving in currents around the larvae were observed. Short cilia covering the apical surface were apparent after 17 hours, and at 18 hours much of the surface of the larvae was covered with cilia. Trochophores were immobile while retained on the gills, although larvae that were suspended in water rotated as a result of ciliary activity.

Apical tuft. At 18 hours, trochophores developed an apical ciliary tuft which appeared as a spike-like projection after 20 hours (Fig. 5). When suspended in water, larvae swam with the tuft pointing in the direction of movement. Trochophores removed from the gills flexed and curled the tuft (Fig. 6). Although the tuft initially appeared to be a single, spike-like structure (Fig. 7), photomicrographs magnified approximately 320 x showed that the tuft was composed of individual cilia (Fig. 8).

Straight-hinged larvae. Straight hinged larvae (veligers) were first observed at 37 hours and became most prevalent 49 hours after spawning (Fig. 9). The spike-like tuft was retained throughout the straight-hinged larval stage, and extended from the velum. As with the trochophores, larvae that were motionless in the gills became motile when manually freed from the gelatinous material covering the gills, and swam with the velum extended in the direction of movement.

Pediveligers. Pediveligers bearing a spike-like tuft on the velum and a ciliated foot were first released from parental clams at 100 hours after spawning. Some pediveligers remained on the gills of parent clams for 125 hours before release. The gelatinous material surrounding the larvae became less thick and less viscous throughout the period of release.

Juveniles. Released pediveligers shed their vela (metamorphosed) to juveniles at 112 hours (about 5 days) after spawning. Of 21 young clams observed at 112 hours, 67% bore only a foot, 24% bore only a velum, and 9% bore a foot and a velum. All larvae were without vela at six days after spawning. Juveniles were characterized by dark spots on the

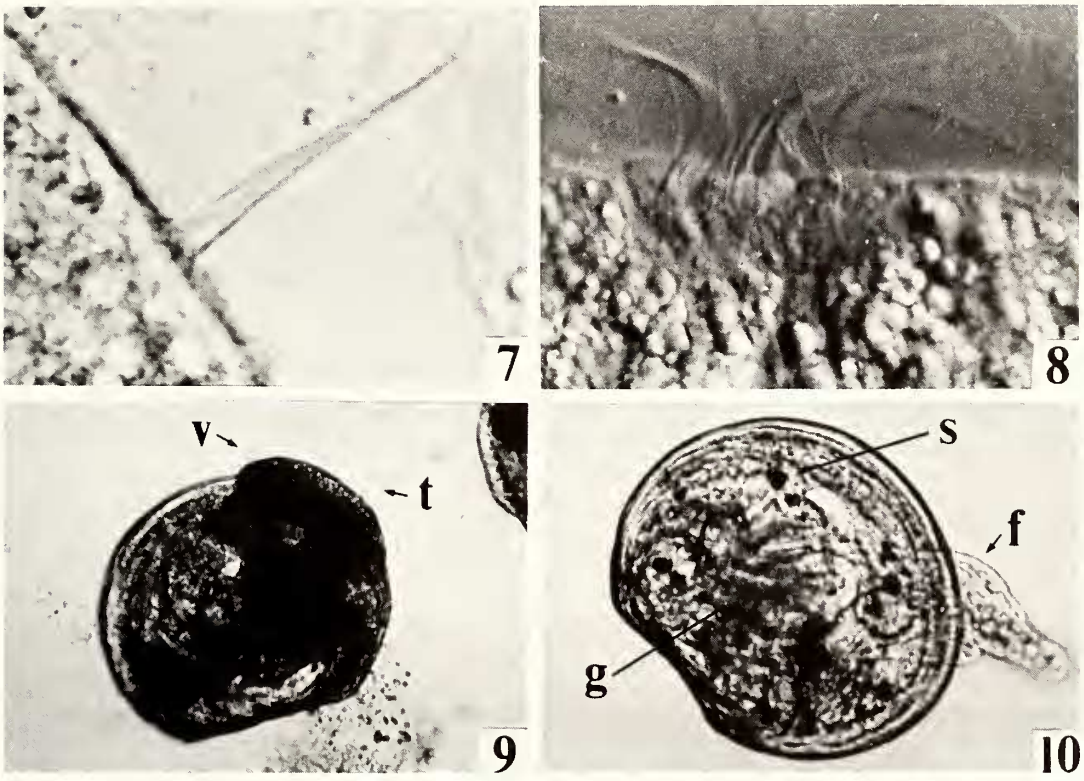

Fig. 7. Apical tuft of *C. fluminea* trochophore. Horizontal field width = 20 μm. **Fig. 8.** Individual cilia of the apical tuft of *C. fluminea* trochophore observed using phase contrast microscopy. Horizontal field width = 40 μm. **Fig. 9.** Straight-hinged (veliger) larva of *C. fluminea* 37 hours after spawning bearing a velum (v) and apical tuft (t). Horizontal field width = 380 μm. **Fig. 10.** Juvenile *C. fluminea* about one month after release from parent. g = gills; s = spot; f = foot. Horizontal field width = 330 μm.

body (Fig. 10), and gills were visible through the shell. The mean shell length of juveniles at metamorphosis was 221 μm (s.d. +/− 10 μm). Shell length increased significantly after one week (two-sample t-test, $t = 10.886$; $P < 0.001$), to 256 μm (s.d. +/− 20 μm). Juveniles became coated with decaying algae and detritus after one to two weeks of culture, and high mortality occurred after three weeks. Remaining juveniles survived for about two months after metamorphosis although little shell growth was observed.

A summary of early development of laboratory-spawned *C. fluminea* is illustrated in Figure 11. The times stated for each developmental stage are based on observations of embryos removed from gills of parent clams, and represent the number of hours after spawning when each stage was first observed, however, as development progressed, gills of parent clams contained brooding embryos at different developmental stages.

Adult clams continued to grow after releasing larvae, and six months after the spawning event, developing eggs and sperm were visible in gonad smears.

FEEDING ACTIVITY OF LARVAE

Particles were visible moving around released larvae as a result of currents produced by the ciliary activity of the velum. Released pediveligers that were exposed to fluorescent latex microspheres contained fluorescent particles within the gut. Larvae brooded on the gills of parent clams showed no gut fluorescence.

DISCUSSION

GROWTH OF ADULT *CORBICULA FLUMINEA*

Culture of *C. fluminea* in the laboratory has been attempted by many investigators using a variety of diets, including strained spinach (Britton and Morton, 1982), and algae, such as *Chlamydomonas, Ankistrodesmus* (Foe and Knight, 1985), *Anabaena, Scenedesmus* (Lauritsen, 1985), and *Chlorella* (Foe and Knight, 1985; Lauritsen, 1985). The diatom *Skeltonema costatum*, fed to clams in the present study, is known to support growth of marine bivalves in intensive culture (Epifanio, 1975). Further investigations of optimal physical and chemical culture conditions as well as nutritional requirements are needed to develop algal diets and culture techniques that support maximum growth of *Corbicula* in the laboratory.

Growth of clams in this study demonstrates that *C. fluminea* is able to tolerate salinities fluctuating from 0 to 8 ppt. Evans *et al.* (1979) reported that *C. fluminea* was able to survive exposures of 10 to 14 ppt salinity without prior

acclimitization, and when clams were allowed to adapt to increasing salinity over a period of 40 to 80 days, they observed that *C. fluminea* could tolerate salinities as high as 24 ppt. Although found primarily in freshwater, sparse populations of *C. fluminea* from the Sacramento-San Joaquin estuary, California, USA were found in 17 ppt salinity (Evans *et al.*, 1979). Mouthon (1981) reported populations of *C. fluminea* from France and Portugal in waters of 30 ppt salinity.

SPAWNING AND LARVAL DEVELOPMENT

Spawning (sperm release) of laboratory clams resulted from a combination of thermal, mechanical, and salinity shocks. Induction of spawning under controlled conditions may be possible in future studies by utilizing one or more of the stimuli mentioned above.

Sperm and egg cells of *Corbicula* spp. have been described in different degrees of detail by several investigators. The biflagellate, conical-headed sperm we observed in laboratory spawned clams were similar to descriptions by Britton and Morton (1982) for sperm from *C. fluminea*, and similar to sperm from *Corbicula* from the Ohio River, Ohio, from Newman, California, and from Phoenix, Arizona (Sinclair and Isom, 1963). Sperm from *C. leana* in Japan described by Cahn (1951) are different in size and shape from sperm from American populations of *Corbicula*, and are characterized by a spherical head 2 μm in diameter that bears a single flagellum 15 μm in length.

The reported size of egg cells of *Corbicula* spp. varies. Eggs from laboratory clams ranged from 120 to 170 μm in diameter. Villadolid and del Rosario (1930) reported immature ova 20 to 160 μm in diameter from *C. manilensis*. Ova of *Corbicula* collected from the Cumberland River, Tennessee, were 50 to 120 μm in diameter (Sinclair and Isom, 1963). Britton and Morton (1982) reported egg cells of 280 μm. Variations in the size of egg cells could be due to species differences or environmental conditions, or the developmental stage of the ova at the time of measurement.

The time sequence of developmental stages (Fig. 11) depicts when each developmental stage was first observed, however there was overlap of consecutive stages. Developmental times may vary with water temperature, and were possibly affected by the removal of larvae from parental gills for observation.

Development of early trochophore larvae began with the formation of lobes lateral to the apex, and the later development of the apical tuft (see also Kraemer and Galloway, 1986). Sinclair and Isom (1963) illustrated apical lobes and a ciliary tuft similar to those we observed in laboratory-raised trochophores, and described a later-staged larvae bearing a "flagellum" which was retained during the pediveliger stage. Veliger larvae of *C. leana*, shown by Cahn (1951) bear a tuft resembling a flagellum. Britton and Morton (1982) described an apical ciliary plate on trochophore larvae, but did not discuss a spike-like tuft that we observed in laboratory larvae.

Scanning electron micrographs of trochophore larvae from bivalves in the family Teredinidae have shown that what had previously been described as the apical "flagellum" on the trochophore is in fact a tuft of cilia (Turner and Boyle, 1974; Boyle and Turner, 1976). Our photomicrographs also show that the "flagellum" at the apical region of the trochophore of *C. fluminea* is composed of many cilia which join and move together, and appear as a spike-like projection in later stages.

Although trochophore larvae were motionless when enveloped in the gelatinous layer on the parental gills, the apical tuft of larvae that were manually freed from the gills flexed from side to side, and the larvae swam actively. The tuft possibly has a sensory function that aids in the orientation of the larvae.

Most species in the genus *Corbicula* that inhabit freshwater brood their larvae, and others, primarily brackish water species, release planktonic larvae without an incubation period (Sinclair, 1971; Morton, 1982). The only freshwater bivalve that releases planktonic larvae is the mussel *Dreissena polymorpha,* which inhabited marine environments until the nineteenth century (Morton, 1958; Russell-Hunter, 1964). Marsupial larval development is an advantage for riverine bivalves since planktonic larvae may be carried downstream away from optimal conditions for survival.

The brooding period of larvae from laboratory clams extended 100 to 125 hours (4 to 5 days) after spawning. Eng (1979) estimated a one month incubation period for *Corbicula* populations from the Delta-Mendota Canal, California, USA, however, estimations of brooding periods based on field observations may be influenced by the method and frequency of sampling. In addition, the brooding period is probably affected by environmental conditions (Eng, 1979).

The developmental stage of larvae that are released from parent clams differs among reports. Release of trochophores and earlier developmental stages has been reported (Heinsohn, 1958, cited in Eng, 1979; Kennedy, 1985), which are possibly aborted broods resulting from environmental stress (Heinsohn, 1958). We observed premature shedding of embryos from a sample of clams that were removed from the aquarium and placed in bowls for observation soon after sperm release occurred, however, the majority of larvae were released at the pediveliger stage 4 days later.

Release of nonswimming pediveliger larvae, as observed in this study, or juvenile clams has been reported elsewhere (Cahn, 1951; Sinclair and Isom, 1963; Eng, 1979; Britton and Morton, 1982; Kennedy, 1985). Larvae of *Corbicula* from the Ohio River are reported to spend a short time in the plankton after release, but are not able to use the velum for swimming (Sinclair and Isom, 1963), and become benthic within 48 hours (Sinclair, 1971). Newly-released clams are well adapted for benthic existence; they bear a strong, ciliated foot and are characterized by advanced anatomical organization compared to other bivalve larvae.

Although juvenile clams grew significantly during the first week after release, they appeared to be in poor condition after three weeks, and heavy mortalities occurred. Attachment of juveniles to sand grains using a mucilaginous attachment thread (Kraemer 1979), was not observed in laboratory-cultured juveniles. Further development of culture

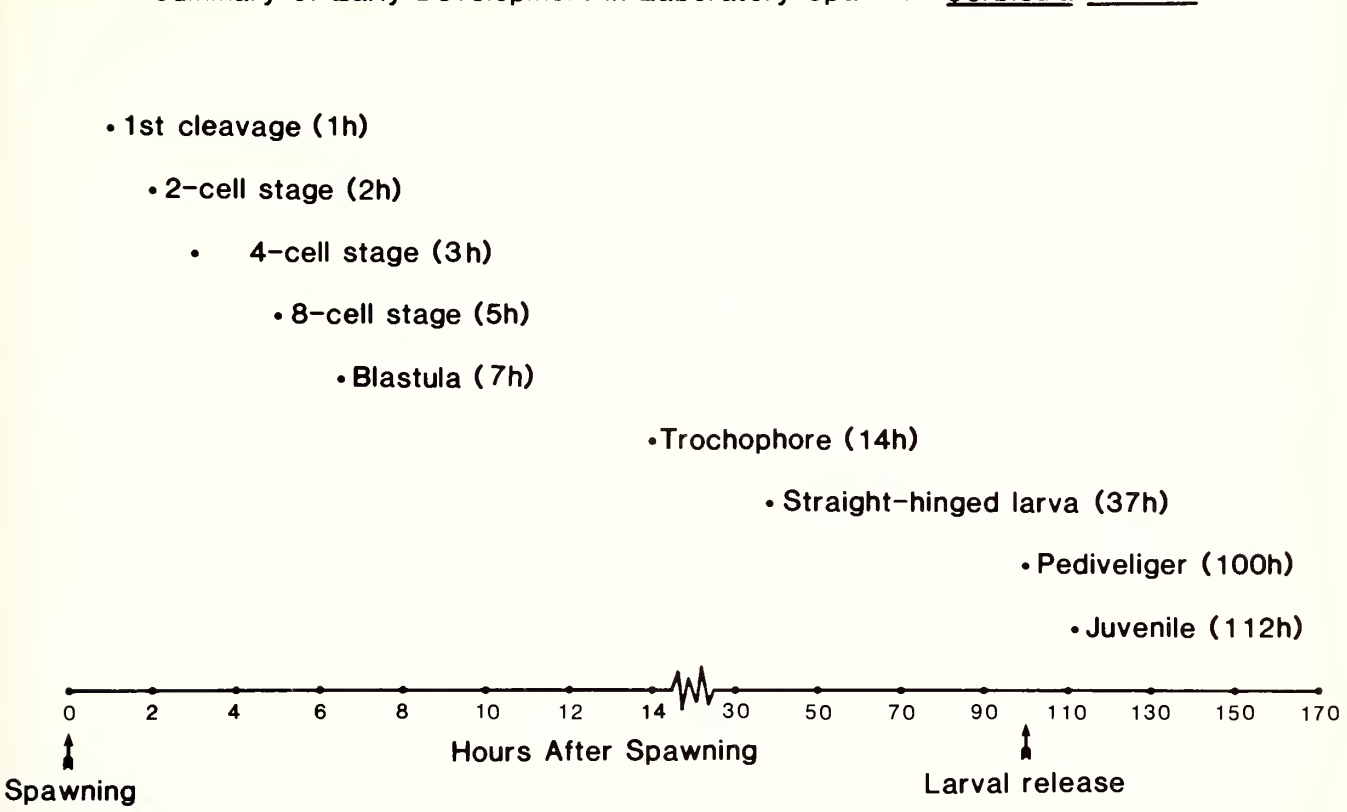

Fig. 11. Summary of early development in laboratory spawned *C. fluminea*.

techniques may enable definition of conditions that induce juvenile attachment.

FEEDING ACTIVITY OF LARVAE

The feeding experiment with fluorescent latex microspheres demonstrated that released pediveliger larvae ingested microspheres, but larvae did not incorporate particles while on the parental gills. More studies on larval feeding activity are necessary to fully understand the nutritive sources for brooding and newly released *Corbicula* (see also Kraemer and Galloway, 1986).

This report is the first account of conditioning and subsequent spawning of *Corbicula fluminea* in laboratory culture. Much more work on laboratory culture of the clams is necessary. Methods to consistently induce release of sperm from conditioned animals will greatly aid in the study of the larval ecology of the clams. Better culture techniques will permit maintenance of clams in the laboratory throughout their entire life cycle and will permit detailed studies on larval development and life history of the organism. Such studies may lead to the development of effective methods for the control of undesirable *Corbicula* infestations.

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